PERITONEAL INFECTION BY CANDIDA ALBICANS. STUDY OF NUMBER AND SIZE OF LIMPHOCYTES AND PHAGOCITIC ACTIVITY OF PERITONEAL MACROPHAGES IN MICE.

Flávio Saad²
Maria Regina R. da Silva³
Hisakazu Hayashi⁴
Olga F. Gompertz⁵
Roberto de Araújo Segreto⁶
Helena Regina Comodo Segreto⁷
Clara Lúcia B. Mestriner⁸
Neil Ferreira Novo⁹
Yara Juliano¹⁰
Hélio Plapler¹¹


SUMMARY: The main purpose of this investigation was to study some aspects of leucocytes (granulocytes and limphocytes) and the phagocytic activity of peritoneal macrophages. In this experiment, which took place at Escola Paulista de Medicina - Universidade Federal de São Paulo - Brazil, it was used twenty female C57BLACK mice. Half of them were submitted to radiation to obtain immunossuppressed animals (Group A - irradiated mice). The other ten mice were not irradiated (Group B - control). The animals were sorted in four subgroups: A-1, A-2, B-1 and B-2. Mice of the groups A-1 and B-1 were injected with saline, and those of subgroups A-2 and B-2, were infected with Candida albicans (ATCC 90029). The resultant data showed significant differences in the number of leucocytes (granulocytes and limphocytes), and in the medium size of limphocytes between irradiated and non irradiated mice. Related to peritoneal macrophages, it was observed that the number of macrophages was lower in irradiated mice and the phagocytic activity was decreased in the irradiated and infected animals.


INTRODUCTION

Although several medical advances have happened in antimicrobial therapy, surgical treatment and surgical intensive care, the number of patients with peritoneal infection remains very high.²⁴,³⁴,³⁷,⁴⁰

Recently some authors have described an important role of Candida albicans in mortality of immunossuppressed patients with peritoneal infection²¹,²²,²³,²⁵,³¹,³⁷,³⁸,⁴⁰

Despite of a great number of clinical reports related to peritoneal Candida infections¹,²,⁵,¹⁸,¹⁹,²¹,²⁵,²⁹,³⁰, experimental research is not so numerous.², ²⁶

1. Summary of Doctoral thesis approved at the Postgraduate Course in Experimental Surgery at the Federal University of São Paulo (UNIFESP) – Escola Paulista de Medicina (EPM).
2. Doctor in Medicine of the UNIFESP-EPM
3. Assistant Professor of Clinical Pathology of the UNIFESP-EPM
4. Full-Professor of Department of Embriology of UNIFESP- EPM
5. Assistant Professor of Micology of UNIFESP- EPM
6. Assistant Professor of Radiotherapy of UNIFESP- EPM
7. Assistant Professor of Radiotherapy of UNIFESP- EPM
8. Assistant Professor of Parasitology of UNIFESP- EPM
9. Assistant Professor of Biostatistics of UNIFESP- EPM
10. Assistant Professor of Biostatistics of UNIFESP- EPM
11. Associate Professor of Experimental Surgery UNIFESP- EPM

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Another issue is the role of leucocytes (lymphocytes and granulocytes) and peritoneal macrophages in diagnosis of Candida peritoneal infections.²,³,⁶,⁷,¹⁷,¹⁹,³⁶,³⁹

The purpose of this work was to investigate some aspects of blood cells (number of leucocytes and size of lymphocytes) and phagocytosis of peritoneal macrophages in mice, infected by *Candida albicans*.

**METHOD**

Twenty C57BLACK female mice weighting between 18 e 22 g were used in this experiment. The animals were distributed in two groups of ten mice each one. These groups were irradiated (A) and not irradiated (B). Four subgroups of 5 animals were obtained. The animals of A-1 and B-1 subgroups were intraperitoneally injected with 1,0 ml of physiologic solution and mice of A-2 and B-2 subgroups received 1,0 ml of a solution of 8,8 (±0,5) x 10⁵ UFC *Candida albicans*.

Exposure to radiation was performed by a 60 Co ALCIAN-II equipment with the following characteristics: single dose of 500 cGy, whole-body exposure, and an absorbed dose of 139,58 cGy.min⁻¹. Animals of A-1 and A-2 subgroups were injected seventy-two hours after the radiation.

Ten days after radiation and seven days after injection the mice were submitted to euthanasia and blood analysis was performed in a Coulter T-890 automatic equipment. Slides were prepared to study the size of the lymphocytes.

Two milliliters of physiological basic solution (PBS) were injected into the peritoneal cavity to recover and prepare the peritoneal macrophages culture.

The number of macrophages were adjusted to 4,5 x 10⁵ and incubated in a Costar Tissue Culture Cluster (24 wells). The macrophages culture remained in 5% CO₂ chamber by twenty hours.

Zymozan** was used to study phagocytic activity and Phagocytic Index (PI) was obtained after common light microscope observations. It was counted the number of macrophages that presented zymozan, and the number of zymozan particles in each macrophage. The PI was expressed by percentage of macrophages with zymozan x the number of zymozan particles in each macrophage.

Statistical analysis were performed by non-parametric tests. KRUSKALL-WALLIS rank variance analysis was used and when there was a statistically significant difference it was completed with multiple comparisons tests. The level of significance was 0,05 or 5% (a² 0,05).

**RESULTS**

There were many important differences between hematologic data from irradiated and non-irradiated mice. The absolute number of leucocytes and the size of lymphocytes were lower in the irradiated groups (1320/1400:5000/3580 by mm³; 5,44/5,47: 6,51/6,00 microns).

It was observed in subgroup A-2 (irradiated and infected mice) a reduced number of granulocytes when compared with the others subgroups (208/276: 627/413 by mm³).

On the other side, the PI was also reduced in the animals of subgroup A-2 when compared to B-1 mice (97:300 or 32:100 - in %).

**DISCUSSION**

The weight of C57BLACK mice were uniform and the known number of *Candida albicans* gave to the experimental design an important caracteristic, an easy possibility of reproduction. *Candida albicans* was chosen based on clinical importance.¹³,²⁰,³⁰,³¹,⁴⁰

The known number of Candida and the period post-infection for the study was suggested by many authors.³,⁴,⁶,⁷,⁹,¹⁰,¹⁵,²⁴,³⁴

GOMPertz (1991)³³ considered a very difficult task to confirm the diagnostic of a *Candida albicans* infection. We could not recover the microorganism in the animals of experiment. Probably, with earlier microbiologic studies this objective would be reached.

There is a tendency to use mice in some experimental peritonitis research.²,¹⁰,²⁶

SEGRETÓ e LUDWIG (1972)⁷ e SEGRETÓ (1992)²⁸ have studied the depletion of leucocytes in blood marrow in C57 BLACK mice, and observed that the period when the depletion was very severe between 48 and 72 hours after the radiation.

The study of number and size of blood cells could be useful for diagnostic of critical infections.⁶,⁷,¹⁷,¹⁹,³⁶,³⁹ Some authors¹¹,¹⁴ observed in mice a number of leucocytes between 4000 and 11400/ mm³. In this study, the averages were between 1320 and 5000/mm³.

(¹) Mc Farland’s 0,5 - Number 90029 ATCC (American Type Collection Culture)
(²) *Saccharomices cerevisae*
Based on the many authors’ data that mice leucocytes were smaller than the human’s. The study of the medium size of mice lymphocytes was 5.44 to 6.51 micra. The reduction of the size of lymphocytes could be related only to radiation or even to radiation and Candida infection in mice.

The phagocytic activity of macrophages can be performed by several methods. It was chosen peritoneal macrophages culture and the Phagocytic Index was calculated as previously described. This indirect method of observation was a adequate model of phagocytic activity evaluation.

Many recent experimental work could be a reflection of the important role of the macrophages and their phagocytic activity in fungal peritoneal infections, specially by Candida. In this way, studying phagocytic activity would be a promising method to improve the survival of patients with peritoneal infection, mainly in those with peritoneal candidiasis.

CONCLUSION

Peritoneal infection by *Candida albicans* in irradiated mice cause a lower number of granulocytes and phagocytosis, while the animals exposed to radiation presents a lower number of leucocytes, lymphocytes and a reduction of the size of lymphocytes.

REFERENCES


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Address reprint request:
Dr. Flávio Saad
Av. Ana Costa, 450/94
Gonzaga - Santos
CEP: 11060-002

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